REMARKS

A. Regarding the Amendments

The present invention provides methods for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA by screening a library containing a plurality of clones obtained from more than one organism. Preferably, each clone contains DNA obtained from a single organism, stably inserting into the clones a substrate that is fluorescent in the presence of the activity of interest, screening the library with a fluorescent analyzer that detects bioactive fluorescence, and identifying clones detected as positive for bioactive fluorescence. Fluorescence is indicative of DNA that encodes a bioactivity or biomolecule.

By the present communication, claims 19 and 30 have been amended to more particularly define Applicants' invention. The amendments to these claims are set forth in the attached "Version With Markings To Show Changes Made" (Exhibit A). Also included is a listing of the claims as they will stand upon entry of the present amendment (Exhibit B). As amended, the claims are supported by the specification and the original claims and add no new matter. In addition, the title of the specification has been amended. The Examiner's suggestion of acceptable alternative language for the title is acknowledged with appreciation. Claims 19-45 are pending.

B. Rejection Under 35 U.S.C. § 112, Second Paragraph

The rejection of claims 19-45 under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention, is respectfully traversed. With specific reference to the recitation "library containing a plurality of clones", Applicants respectfully disagree with the Examiner's assertion that this recitation renders claim 19 (and claims 20-45 dependent therefrom) indefinite. The specification discloses that the libraries referred to in claim 19 can

contain greater than 10^8 members and can represent single organisms or the genomes of over 100 different microorganisms, species, or subspecies (see page 16, lines 14-16). Thus, when claim 19 is read in view of the specification, those skilled in the art would readily understand what is meant by a "library containing a plurality of clones".

With specific reference to claim 20, it is respectfully submitted that this claim is clear as written. This claim further defines the method of claim 19 by reciting an additional method step, wherein the step comprises obtaining DNA from a clone that is positive for an enzymatic activity of interest. In addition, a wide variety of enzymatic activities are set forth in the specification (see, e.g., pages 41-46). Thus, when claim 20 is read in view of the specification, those skilled in the art would readily understand the method step comprising "obtaining DNA from a clone that is positive for an enzymatic activity of interest".

With specific reference to the term "biopanning", Applicants respectfully disagree with the Examiner's assertion that this recitation renders claim 39 indefinite. This term is explicitly defined in the specification (see, e.g., page 33, line 20 to page 34, line 3). Thus, when claim 39 is read in view of the specification, those skilled in the art readily understand what is meant by "bioipanning the expression library prior to contacting with the substrate".

Finally, Applicants respectfully submit that the amendment to claim 19 presented herein renders the alleged lack of clarity issue moot with respect to claim 42. Thus, for all of the reasons set forth above, it is respectfully submitted that the rejection of claims 19-45 under 35 U.S.C. § 112, second paragraph is not properly applied. Accordingly, reconsideration and withdrawal of the rejection are respectfully requested.

C. Rejection Under 35 U.S.C. 102(b)

The rejection of claims 19-21, 35, and 37 under 35 U.S.C. 102(b), as allegedly being anticipated by Abeijon, et. al. (Proc. Natl. Acad. Sci. USA 93:5963-5968, 1993), is respectfully

traversed. Applicants' invention, as defined by claim 19, distinguishes over Abeijon by requiring "a method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA comprising:

a) contacting a bioactive substrate that is fluorescent in the presence of the bioactivity or biomolecule of interest with a library containing a plurality of clones containing DNA from at least one organism;

b) screening the library with a fluorescent analyzer that detects bioactive fluorescence, and

c) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule of interest."

Each clone of the library contains DNA from at least one organism within the mixed population. Thus, Applicants' method involves cloning of individual genes or groups of genes (e.g., pathways) obtained from an organism, into a host cell. The clones in Applicants' library include host cells containing DNA (e.g., single genes or pathways) obtained from an organism that may encode one gene product or more than one gene product.

In contrast, Abeijon does not disclose a method as defined by claim 19. Indeed, Abeijon is silent regarding preparation of a library of naturally occuring genes or gene pathways in which each clone may contain any type of DNA and wherein the DNA in each clone is obtained from an organism from a mixed population of organisms. Therefore, Abeijon fails to teach each and every element of Applicants' method as defined by claim 19. Accordingly, reconsideration and withdrawal of the rejection of claims 19-21, 35, and 37 under 35 USC § 102(b) are respectfully requested.

D. Double Patenting

The rejection of claims 19-45 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-5, 8-11, 15, 16, 18, 19, and 21 of U.S. Pat.

No. 6,174,673 is acknowledged. This issue has been addressed by the Terminal Disclaimer which accompanies this response.

A check in the amount of $255.00 ($200.00 for the two-month extension of time fee and $55.00 for the Terminal Disclaimer fee) is enclosed in connection with the filing of this paper. The Commissioner is hereby authorized to charge any other fees associated with the filing submitted herewith, or credit any overpayments to Deposit Account No. 50-1355. A duplicate copy of the Transmittal is enclosed.

In view of the amendments and the above remarks, it is submitted that the claims are in condition for allowance and a notice to that effect is respectfully requested. The Examiner is invited to contact Applicants' undersigned representative if there are any questions relating to this application.

Respectfully submitted,

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Enclosures: Exhibit A
Exhibit B

Exhibit A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

19. (Amended) A method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA comprising:

a) contacting a bioactive substrate that is fluorescent in the presence of the bioactivity or biomolecule of interest with a library containing a plurality of clones containing DNA from **[more than]** at least one organism;

b) screening the library with a fluorescent analyzer that detects bioactive fluorescence, and

c) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule of interest.

30. (Amended) The method of claim 19, wherein the bioactive substrate comprises staining reagent C12-fluorescein-di-D-galactopyranoside (C12FDG).

Exhibit B

CLAIMS UPON ENTRY OF THE AMENDMENT

19. A method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA comprising:

a) contacting a bioactive substrate that is fluorescent in the presence of the bioactivity or biomolecule of interest with a library containing a plurality of clones containing DNA from at least one organism;

b) screening the library with a fluorescent analyzer that detects bioactive fluorescence, and

c) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule of interest.

20. The method of claim 19, further comprising obtaining DNA from a clone that is positive for an enzymatic activity of interest.

21. The method of claim 20, wherein the enzymatic activity of interest is from an enzyme selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

22. The method of claim 19, wherein the library is generated in a prokaryotic cell.

23. The method of claim 22, wherein the library contains at least about 2×10^6 clones.

24. The method of claim 22, wherein the prokaryotic cell is gram negative.

25. The method of claim 19, wherein the clones are encapsulated in a gel microdrop.

26. The method of claim 19, wherein the analyzer screens up to about 15 million clones per hour.

27. The method of claim 19, wherein the clones are extremophiles.

28. The method of claim 27, wherein the extremophiles are thermophiles.

29. The method of claim 27, wherein the extremophiles are hyperthermophiles, psychrophiles, halophiles, psychrotrops, alkalophiles, or acidophiles.

30. The method of claim 19, wherein the bioactive substrate comprises staining reagent C12-fluorescein-di-D-galactopyranoside (C12FDG).

31. The method of claim 19, wherein the bioactive substrate comprises a lipophilic tail.

32. The method of claim 19, wherein the clones and substrates are heated to enhance contacting of the substrate with the clones.

33. The method of claim 32, wherein the heating is to a temperature of about 70°C.

34. The method of claim 32, wherein the heating is for about 30 minutes.

35. The method of claim 19, wherein the fluorescent analyzer comprises a fluorescence activated cell sorting (FACS) apparatus.

36. The method of claim 20, wherein the enzymatic activity of interest encoded by the DNA is stable at a temperature of at least about 60°C.

37. The method of claim 19, wherein the library is an expression library.

38. The method of claim 20, wherein the enzymatic activity of interest encoded by the DNA possesses enhanced enzymatic activity of interest compared to that of a wild-type enzyme.

39. The method of claim 19, wherein the method further comprises biopanning the expression library prior to contacting with the substrate.

40. The method of claim 19 further comprising obtaining DNA from a clone identified in step c) that is positive for an enzymatic activity of interest and comparing the enzymatic activity of a DNA expression product from the clone with that obtained from such a clone into whose DNA at least one nucleotide mutation has been introduced, wherein a difference in enzymatic activity is indicative of the effect upon the enzymatic activity of interest caused by introduction of the at least one nucleotide mutation.

41. The method of claim 19, wherein the bioactivity encoded by the DNA possesses the bioactivity of interest at a temperature at least 10°C below the temperature of optimal activity of the bioactivity encoded by the wild-type DNA.

42. The method of claim 19, wherein each clone contains DNA obtained from a single organism.

43. The method of claim 19, wherein the library is a multispecies library.

44. The method of claim 43, wherein the library is generated from a mixed population of uncultured organisms.

45. The method of claim 43, wherein the library is generated from isolates.

46. The method of claim 40, wherein the mutation is introduced by error-prone PCR, oligonucleotide directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis and exponential ensemble mutagenesis.